

Remarks/Arguments:

1. Claims 1-8 and 20 remain pending.

2. Claim 1 is being amended to expressly incorporate the description of the claim term "capture antibody" from the specification. The amendment makes no substantive change to the subject matter claimed. Support for the amendment to claim 1 is found in the specification as originally filed, for example, at page 4, lines 19-23, which specifically describes what is meant by the claim term "capture antibody" as that which is now specifically recited in the language being added by amendment. Accordingly, as the Examiner will appreciate, no new matter is added by this amendment.

3. Objections maintained.

Information Disclosure Statement - Applicants acknowledge that the Examiner, as discussed in Item 2 of the Action, has only considered references cited by the Examiner on form PTO-892 or by Applicants on form PTO-1449 and has not considered other publications referenced in the specification but not otherwise cited in the forms discussed above.

Specification - In accordance with Item 4 of the Action, Applicants submit amendments to the specification hereinabove to properly note and respect the proprietary nature of trademarks.

4. Claims 1-8 and 20 stand rejected, under 35 U.S.C. § 112, second paragraph, as indefinite on the grounds that "it is unclear if Applicant intends to measure CDK activity or simply antibody detection of a retinoblastoma protein in a given sample." In response to Applicants' previous assertion that claimed invention measures CDK activity in a sample because the specific CDK-phosphorylated Rb residues are specifically recognized by the claimed capture antibody, the Examiner asserts that "the claims do not recite specific CDK phosphorylation sites specifically recognized by the capture antibody." While Applicants respectfully disagree, and provide that the specific phosphorylation sites of Rb are incorporated into the claim by means of the specification's express description of "capture antibody" (see specification at page 4, lines 19-23), Applicants propose amending claim 1 (the limitations of which are incorporated into all other pending claims) to even more clearly provide that which Applicants' regard as the invention, and to expressly recite the description of a capture antibody as an antibody that specifically recognizes Rb protein phosphorylated at a residue phosphorylated by a CDK: Ser249, Thr252, Thr356, Ser612, Ser780, Ser807, Ser811, or Thr821. Measurement of the anti-Rb antibody present in the capture antibody-Rb-primary antibody complex measures CDK activity by means of quantitating the CDK phosphorylated Rb. Applicants note that, while the anti-Rb antibody can recognize Rb in a phosphorylation-independent manner, only Rb phosphorylated at specific residues by CDK is present in the capture antibody-Rb-primary antibody complex. Therefore, quantitation of the anti-Rb antibody is a measure of phosphorylated Rb and CDK activity with respect to phosphorylation at the specifically named residues.

Therefore, entry of the amendment hereinabove, and reconsideration of the Office Action mailed July 29, 2003 are respectfully requested.

5. Obviousness

Claims 1, 4-7, and 20 stand rejected, under 35 U.S.C. § 103, for obviousness over Wen et al. (hereinafter "Wen") in view of Juan et al. (hereinafter "Juan"). The Examiner contends that Applicants' rebuttal of this

rejection in the response of May 5, 2003 is not found persuasive because the limitation relied upon to distinguish the prior art, use of a capture antibody specific for CDK-phosphorylated residues on the Rb protein, is "not recited in the rejected claim(s)." While Applicants respectfully disagree with this assertion (as discussed above in paragraph 4), Applicants note that the proposed amendment to claim claim 1 provided herein expressly recites the definition of a capture antibody as an antibody that specifically recognizes Rb protein phosphorylated at a residue phosphorylated by a CDK: Ser249, Thr252, Thr356, Ser612, Ser780, Ser807, Ser811, or Thr821. Wen is merely an assay for detecting total Rb. Wen does not disclose or suggest the specific Rb residues targeted for assessment of CDK activity by the present invention. Furthermore, Wen neither discloses nor suggests the existence of the CDK "capture antibodies" used in the present claimed invention which specifically recognize Rb phosphorylated at these specific CDK-mediated residues, or that such antibodies could be successfully used to capture CDK-phosphorylated Rb as a means of assessing CDK activity in combination with an anti-Rb antibody. Rather, Wen merely uses two different antibodies that recognize Rb in a phosphorylation-independent manner to measure total Rb.

The deficiencies of Wen are not cured by Juan. Juan discloses an assay for monitoring Rb phosphorylation by incubating cells with dual fluorochrome-tagged antibodies. While one antibody assesses total Rb, a second antibody is specific for underphosphorylated Rb. Rb phosphorylation is assessed by comparing the dual fluorochrome signals that represent either total Rb or underphosphorylated Rb. With respect to the phosphorylation-dependent antibody, Juan merely discloses an antibody completely distinct from the "capture antibody" of the presently claimed invention. The Juan phosphorylation-related antibody recognizes an underphosphorylated Rb, rather than the specifically CDK-phosphorylated Rbs recognized by the capture antibody of the present claimed invention. Furthermore, Juan uses its antibodies to measure dual readouts rather than a single readout in an ELISA-based dual antibody-complex assay. The present claimed invention, rather than relying on dual readouts to assess the CDK-mediated Rb phosphorylation, directly measures only the second anti-Rb primary antibody. This is correlated with, but not a direct measure of, the capture antibody present because the only Rb present for the anti-Rb primary antibody to measure is indeed CDK-phosphorylated Rb following isolation of the capture antibody-Rb complex from the remainder of the sample, as recited in Claim 1, step (ii).

Either alone, or in combination with the above-discussed Wen, Juan in no way discloses or suggests that Rb is phosphorylated by CDKs at the residues recognized by the capture antibodies of the present invention, that antibodies specific for detecting CDK at these residues exist or can be made, or that such antibodies could be successfully used as a capture antibody, in combination with an anti-Rb primary antibody, as in the claimed invention, to detect CDK phosphorylation at these specific sites using an ELISA based format. Accordingly, entry of the amendment hereinabove and reconsideration of the Office Action mailed July 29, 2003 are respectfully requested.

6. Obviousness

Claims 2 and 3 stand rejected, under 35 U.S.C. § 103, for obviousness over Wen in view of Juan and further in view of Watanabe et al. (hereinafter "Watanabe"). Applicants respectfully note that the same deficiencies described above with respect to Wen and Juan apply to the rejection of claims 2 and 3. Turning to Watanabe, Applicants submit herewith corroborative documentary evidence in support of the Declaration of Barbara A. Foster and Farzan Rastinejad (previously submitted May 5, 2003) which states that Applicants' invention was conceived and reduced to practice prior to July, 1999, and, thus, prior to the publication of Watanabe. This document, sent by facsimile transmission from Pfizer's New York Patent Department on June 10, 1999, details the conception and reduction to practice of the CDK assays of the present invention and was attached to a request for filing a patent application submitted by inventor Farzan Rastinejad. In view of Applicants' previously submitted Declaration and the supporting document enclosed herewith, Applicants respectfully request entry of the amendment hereinabove and reconsideration of the Office Action mailed July 29, 2003.

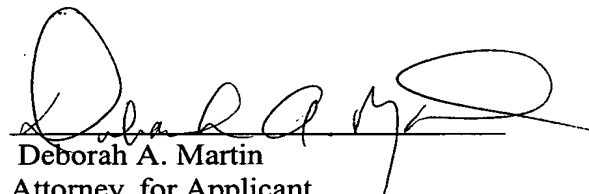
7. Obviousness

Claim 8 stands rejected, under 35 U.S.C. § 103, as obvious over Wen, in view of Juan, and further in view of Maggio. Applicants respectfully note that the same deficiencies described above with respect to Wen and Juan apply to the rejection of claim 8. These deficiencies in Wen and Juan are not cured by Maggio which merely discloses a general discussion of ELISA-based assays using a solid phase/test plate. Maggio alone, or in combination with Wen or Juan, does not disclose or suggest the particular capture antibodies used in the present claimed invention, or their successful use in the ELISA-based assay of the present invention. Therefore, entry of the amendment hereinabove and reconsideration of the Office Action mailed July 29, 2003 are respectfully requested.

8. Applicant believes that the amendments hereinabove place the Application in condition for immediate allowance. Therefore, entry of the amendment hereinabove, and reconsideration of the Office Action mailed July 29, 2003 are respectfully requested. Such prompt and favorable action is earnestly solicited.

Respectfully submitted,

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Appendix A attached

Invention:

This patent concerns a method for measuring the activity of cell cycle kinases, CDK2 and CDK4, within cells with the purpose of identifying compounds that inhibit these enzymes. Such compounds could have utility for inhibiting tumor growth.

Cyclin dependent kinases (CDK2 and CDK4) phosphorylate the retinoblastoma (RB) protein on specific peptide sequences in vivo. Rabbit polyclonal antibodies were generated to recognize peptides derived from the RB protein that contain the specific phospho-serine or phospho-threonine residues that are phosphorylated by CDK2 or by CDK4. A 96-well ELISA assay was invented that uses these antibodies to quantitatively detect the presence of phospho-specific epitopes on cellular RB protein in T47D breast carcinoma cells (ATCC HTB-133)

Methods:

T47D cells are seeded in 96-well plates at 9,000 cells/200 ul/well in DMEM 10% fetal bovine serum (FBS) supplemented with 2 mM L-glutamine, 100 U/ml penicillin and 100 ug/ml streptomycin (P/S/G). For analysis of CDK2 specific phosphorylation, cells are starved in 200 ul of 0.1% BSA in DMEM for 24 hours to synchronize them in the G0 phase of the cell cycle. Cell synchronization by starvation is not required for analysis of CDK4 phosphorylation. The CDK2 and CDK4 kinase activity is stimulated in cells by addition of 10% FBS for 20 to 24 hours. Cells are treated with test compounds (inhibitors of CDK2 or CDK4) during this period.

Nunc F96 MaxiSorp 96-well capture plates are coated overnight at 4°C with the appropriate CDK2 or CDK4 phospho-Rb-specific antibody (refer to materials) at 10 ug/ml, 100 ul/well, in carbonate buffer, pH 9.2. Cell lysates are prepared by first rinsing the wells 3 times with cold Tris buffered saline, then adding 120 ul of cold lysis buffer per well. Plates with lysis buffer are maintained at 4°C for 1 hour. ELISA plates are rinsed 2 times with imidazol wash buffer and blocked with skim milk blocker (0.05% Non-fat dry milk in 20 mM Tris, pH 7.4, 150 mM NaCl, 0.1% Tween 20. After 1 hour, blocker was aspirated from the capture plate and 100 ul of lysate was transferred to each well. The plates are placed on a shaker for 2 hours at room temperature. Plates are then washed 6 times with imidazol wash buffer followed by addition of 100 ul of 1° antibody (α -Rb monoclonal antibody (Pharmingen #14001A) @ 2 ug/ml in Casein Blocker TBS/0.1% Tween-20) and placed on the shaker for another 2 hours. Plates are again washed 6 times with imidazol buffer, and 100 ul of 2° antibody (Donkey α -Mouse-AP (Jackson ImmunoResearch Laboratories, Inc. #715-055-150) @ 1:2000 in Casein TBS/0.1% Tween-20 Blocker) is added with shaking for 1 hour. A final 6 washes are employed and 100 ul of Tropix CDP-Star with Sapphire II substrate is added to each well. Substrate development proceeds for 20 minutes, after which substrate is transferred to opaque 96-well plates and luminescence is read in a Dynatech Microplate luminometer.

Materials:

Nunc F96 MaxiSorp 96-well plates (VWR #62409-002) for the ELISA

Capture antibodies were obtained from three sources: (1) α -Rb P-Ser780 (MBL #555) was purchased from USA-MBL International. (2) α -Rb P-Ser807/Ser811 was purchased from New England Biolabs, Inc. (3) Several polyclonal antibodies were generated by for us by Quality Controlled Biochemicals, Inc., who prepared antigenic phosphopeptides corresponding to the following eptopes on the retinoblastoma protein: Rb P-Ser780, Rb P-Ser807/Ser811, Rb P-Ser249/Thr252, Rb P-Ser612, Rb P-Thr356, and Rb P-Thr821.

Capture antibody for CDK4: α -Rb P-Ser780 (MBL #555) or QCB, Inc. @ 10 ug/ml in 0.05M Carbonate-Bicarbonate Buffer, pH 9.6 (Sigma #C-3041).

Capture antibody for CDK2: α -Rb P-Ser807/Ser811 (available commercially from New England Biolabs, Inc. Cat. No. 9308L or additional antibodies developed for us by QCB, Inc. used @ 10 ug/ml in 0.05M Carbonate-Bicarbonate Buffer, pH 9.6 (Sigma #C-3041).

Wash buffer: Imidazol wash buffer (KPL #50-63-01)

Block buffer for use before adding lysate: 0.05% Non-fat dry milk in 20 mM Tris, pH 7.4, 150 mM NaCl, 0.1% Tween 20

Casein buffer for Antibody Dilutions: 1% Casein in TBS + 0.1% Tween-20 (1% Casein TBS, Pierce #37532).

T47D Breast carcinoma cells (ATCC HTB-133)—lysates prepared in HEPES NP-40 buffer with protease inhibitors.

Lysis buffer: 50 mM HEPES, 0.1% NP-40, 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 1 mM DTT, 50 ug/ml Aprotinin, 1 mg/ml Pefabloc.

1° Antibody: α -Rb Monoclonal antibody (Pharmingen #14001A) @ 2 ug/ml in Casein Blocker TBS/0.1% Tween-20—use 38 ul/9.5 ml.

2° Antibody: Donkey α -Mouse-AP (Jackson ImmunoResearch Laboratories, Inc. #715-055-150) @ 1:2000 in Casein TBS/0.1% Tween-20 Blocker (Use 9.5 ul/19 ml).

Substrate: CDP-Star with Sapphire II (Tropix #MS100RX).